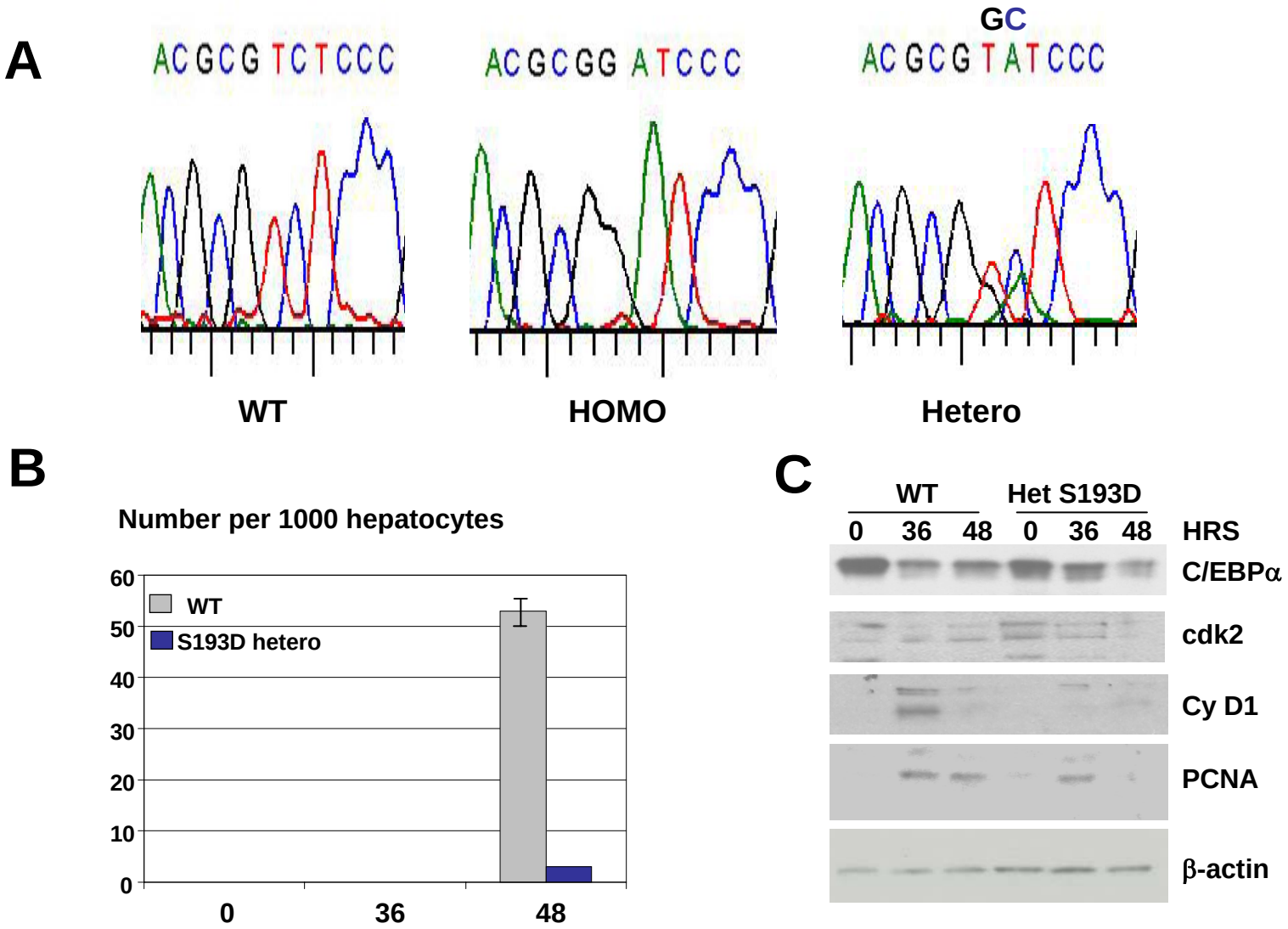
Wang et al Supplemental figures



Supplemental figure 1. S193D-C/EBPα inhibits liver proliferation in heterozygous C/EBPα-S193D mice.
A. Sequencing of PCR products from WT, homozygous and heterozygous S193D mice. B. Examination of mitotic figures in livers of WT and S193D heterozygous mice after partial hepatectomy. Number of mitotic figures was calculated per 1000 examined hepatocytes at 0, 36 and 48 hours after PH. **C. Expression of cell cycle proteins is inhibited in livers of heterozygous S193D mice.** Nuclear extracts were analyzed by Western blotting with antibodies to proteins shown on the right. The membrane was re-probed with β–actin to verify protein loading**.**

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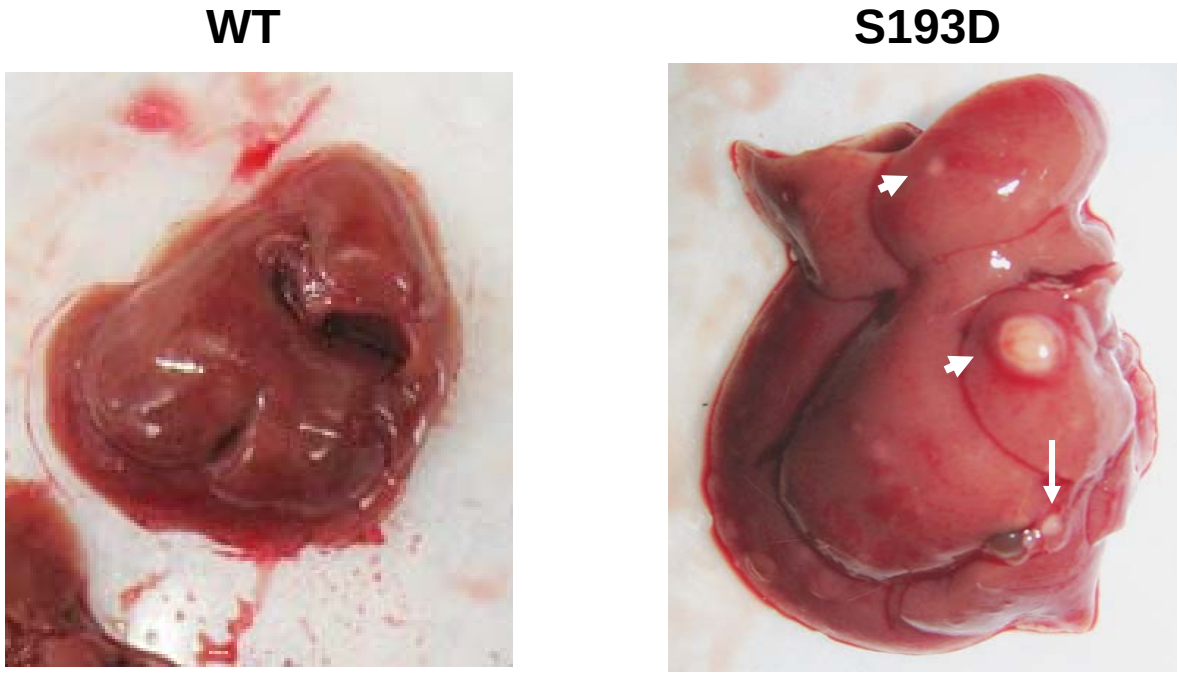

Supplemental Figure 2. Liver tumors in S193D mice 25 weeks after DEN/PB treatments. Arrows show three big tumor nodules observed in livers of S193D mice.

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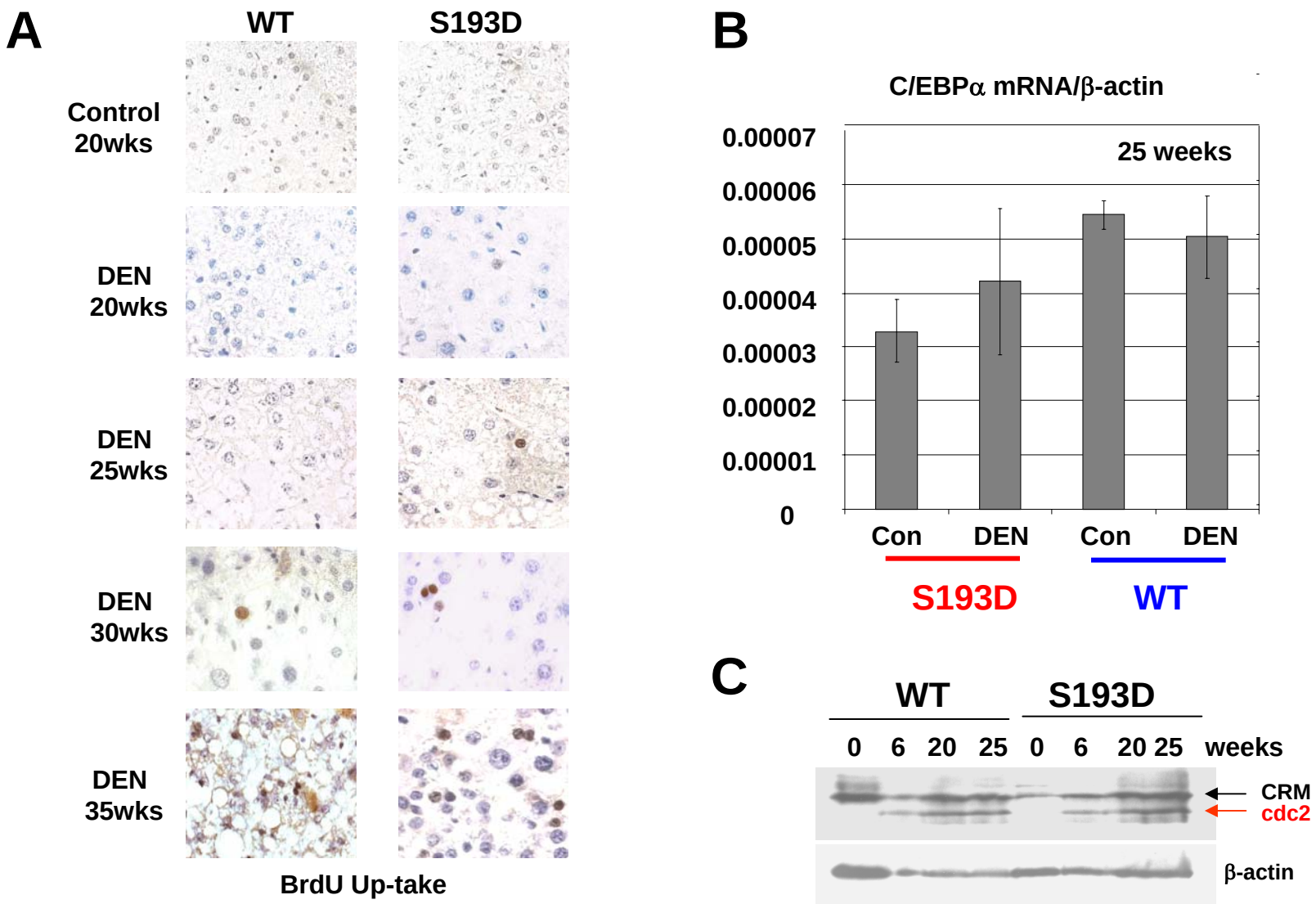

Supplemental figure 3.
A. Images of BrdU staining of the livers during DEN/PB-mediated carcinogenesis in WT and S193D mice.
B. Levels of C/EBPα mRNA determined by Real Time-PCR.
C. Cdc2 is elevated in WT and S193D mice during DEN/PB-mediated carcinogenesis. Western blotting was performed with nuclear extracts isolated at different time points after DEN injection. CRM; cross-reactive molecule.

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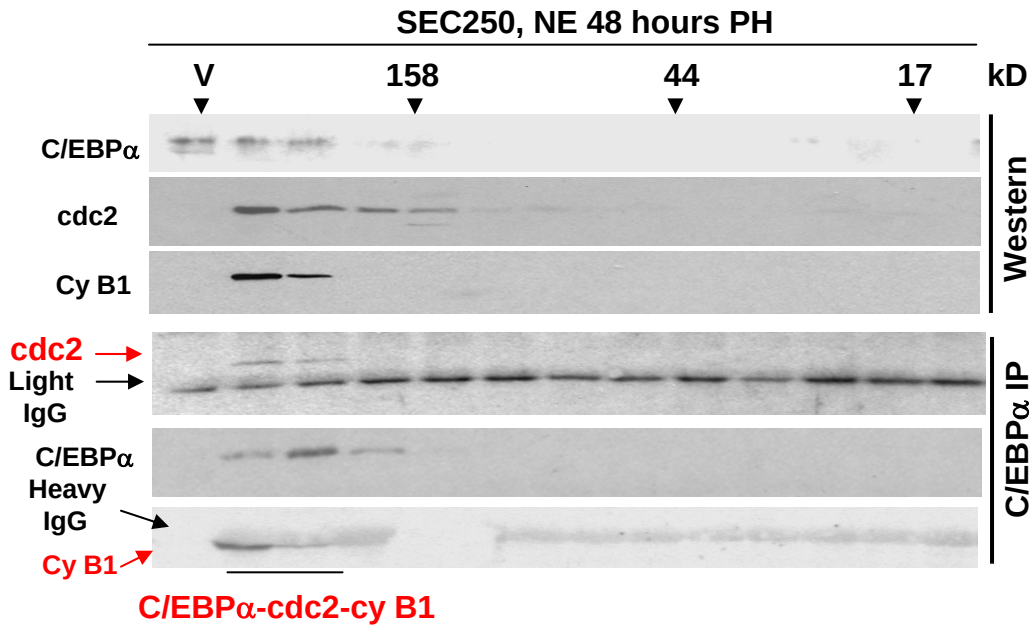

Supplemental figure 4. Analysis of C/EBPα-cdc2 complexes in nuclear extracts isolated from livers at 48 hrs after PH by HPLC-based size exclusion chromatography. Nuclear extracts of 48 hours PH livers were separated by size exclusion chromatography and the fractions were analyzed by Western blotting. Positions of size exclusion markers are shown on the top. **C/EBPα–IP**: C/EBPα was immunoprecipitated from the fractions and IPs were probed with Abs to cdc2 and to cyclin B1.

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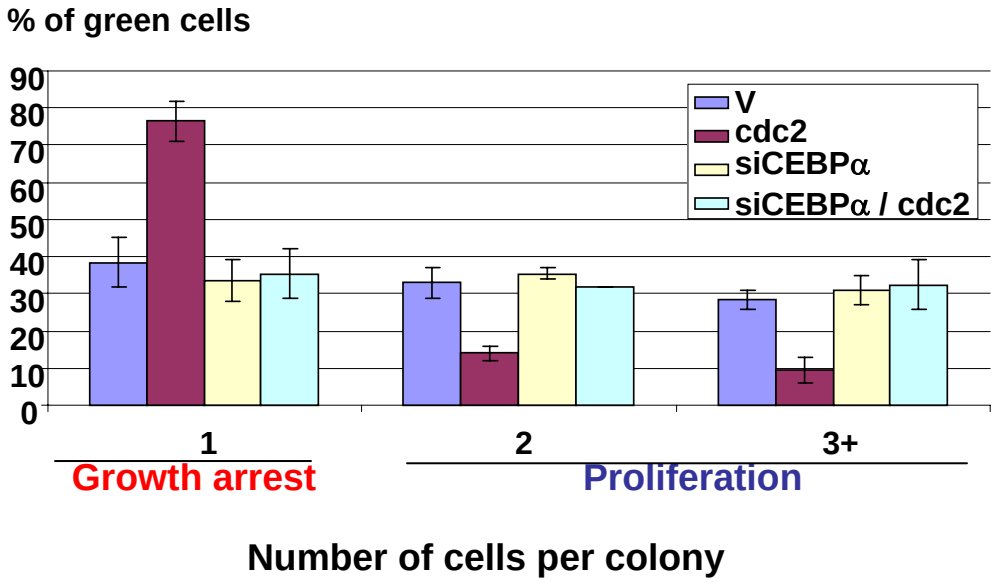

Supplemental figure 5. Cdc2 inhibits proliferation of mouse Hepa 1-6 cells via restoration of growth inhibitory activity of C/EBPα. Hepa 1-6 cells were transfected with pAdTrack-cdc2 and with pAdTrack-cdc2 +siRNA to C/EBPα. Percentage of single cells (growth arrest) and proliferating cells was calculated.

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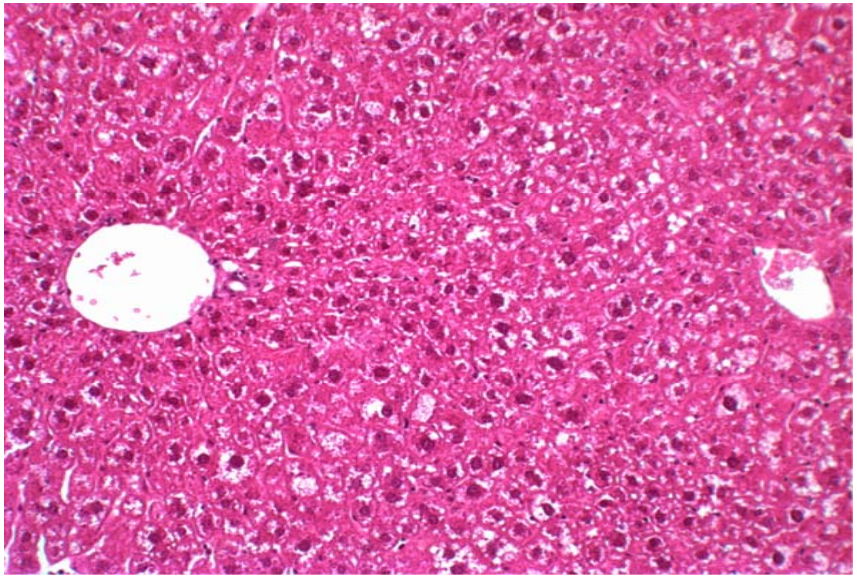
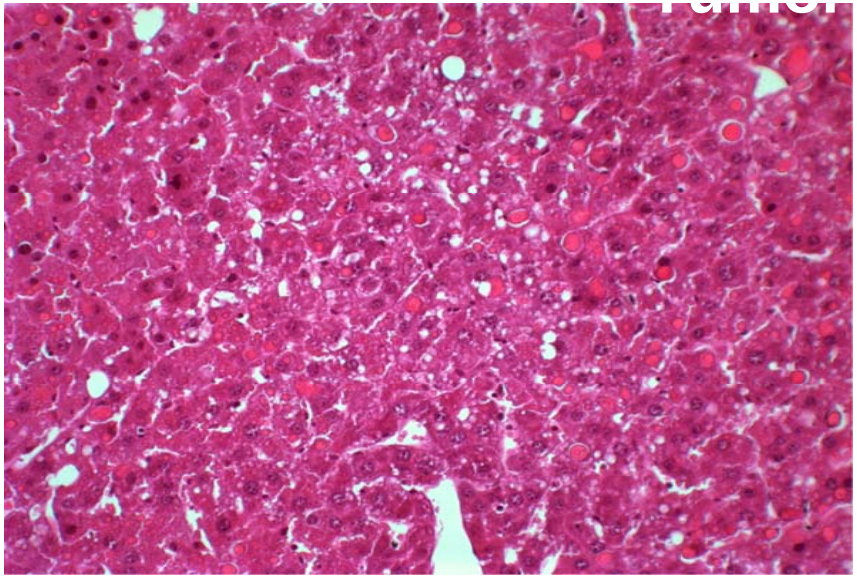
A

B





Supplemental Figure 6. Old animals have increased liver cancer. A. A typical picture of liver tumor in wild type mouse at 22 mo in which a large tumor replaces half the liver**. B. Top.** Representative region of the non-tumoral portion containing a portal tract on the left and terminal hepatic vein to the right, with uniformly unremarkable hepatocytes having plentiful cytoplasmic glycogen. There is slightly variable nuclei size. **Bottom**. Representative region of the large tumor in which the normal lobular architecture and portal tracts, including bile ducts, are not present. Hepatocytes are highly variable, with most containing cytoplasmic fat vacuoles or large eosinophilic protein globular inclusions and having hyperchromatic nuclei, with occasional atypical mitoses .

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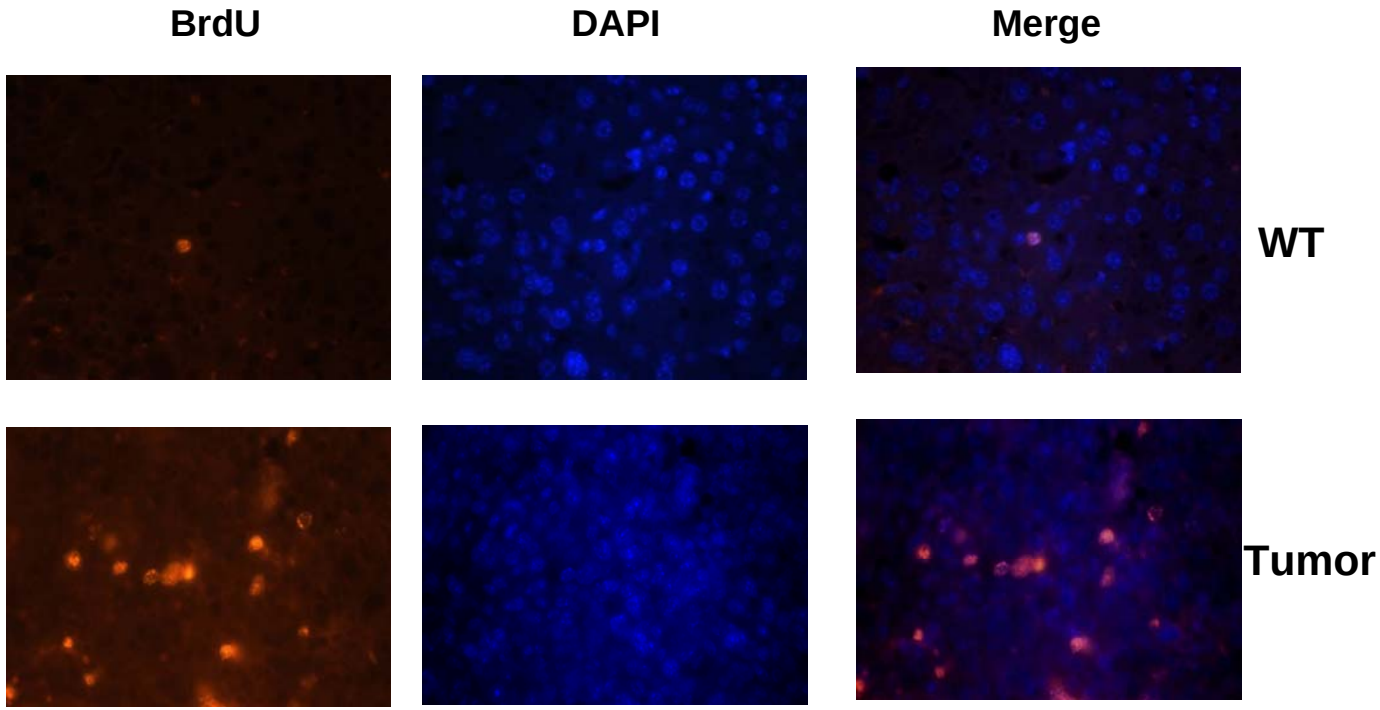

Supplemental figure 7. BrdU up-take in the control and tumor sections of the livers from old mice. BrdU was injected into mice 4 hours before animals were sacrificed. The livers were stained with antibodies to BrdU and with DAPI.